



Stothart, G., Smith, L. J., & Milton, A. (2020). A rapid, neural measure of implicit recognition memory using fast periodic visual stimulation. *NeuroImage*, 211, [116628].
<https://doi.org/10.1016/j.neuroimage.2020.116628>

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A rapid, neural measure of implicit recognition memory using fast periodic visual stimulation

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ARTICLE INFO

Keywords:

Recognition memory
Oddball
Steady-state
EEG
Implicit
Visual evoked potentials
Fast periodic visual stimulation

ABSTRACT

Fast periodic visual stimulation (FPVS) has recently emerged as a powerful new tool in cognitive neuroscience. Capable of measuring a range of cognitive functions in single subjects in just minutes of recording time, it has been adapted to measure visual, semantic and linguistic processing. We present a new adaptation of the FPVS approach to measure recognition memory via old/new contrasts. Twenty one subjects (23 (\pm 6) yrs, 7 males) completed an FPVS-oddball paradigm that assessed their spontaneous ability to differentiate between rapidly presented images on the basis of a pre-FPVS encoding task, i.e. oddball stimuli were only defined by the subject's experimentally induced memory of them. A clear oddball detection response reflecting recognition memory was observed within one minute of EEG recording time, simply through the passive viewing of stimuli, i.e. subjects received no task instructions and provided no behavioural response. Performance on a subsequent behavioural recognition task showed high levels of recognition of the oddball stimuli. As such, the FPVS approach returned an objective, non-verbal measure of recognition memory in just one minute of recording time, free from the confounds of behavioural recognition tasks. This finding reinforces the adaptability of the FPVS approach for the examination of higher-level cognition and provides a new method for the neural measurement of recognition memory.

1. Introduction

Reliable objective measures of cognition are key experimental and clinical goals of cognitive neuroscience. Electroencephalography (EEG) provides a non-invasive objective measure of neural activity that has been used to examine a wide range of cognitive processes from perception through to higher order cognition (Polich et al., 2008; Pratt, 2011), as well as functional network properties (Khanna et al., 2015; Stam, 2014). However despite decades of experimental progress the clinical use of EEG remains remarkably limited, with current common clinical uses restricted to identifying epileptiform activity (Noachtar and Rémi, 2009), studying sleep disorders (Petit et al., 2004) and measuring gross spectral changes in disorders of consciousness (Brenner, 2005).

One of the major barriers to the translation of experimental EEG findings into viable clinical tools has been the reliability of measures at the level of the individual. To obtain sufficient Signal to Noise Ratio (SNR) using traditional Event Related Potential (ERP) techniques, for example, subjects must typically complete hundreds or thousands of experimental trials, which are averaged together in order to identify

experimental effects. Differences observed in group grand average waveforms may be absent in any one individual's average, and there are further sources of bias and inter-experiment variability in the selection of electrodes, time windows and quantification methods that further hamper the reliability and reproducibility of results (Kappenman and Keil, 2017).

Recently a new technique has emerged that solves many of the SNR and analysis bias related problems of ERPs and may be the foundation for viable clinical EEG measures of cognition. First demonstrated by Heinrich et al. (2009) and developed extensively by Rossion et al. (e.g. Alp et al., 2016; Liu-Shuang et al., 2016; Rossion et al., 2015) Fast Periodic Visual Stimulation (FPVS) involves frequency tagging standard and oddball stimuli in a classic oddball paradigm. Standard stimuli are presented at a fast rate, typically around 6 Hz, with oddball stimuli embedded in the train of standard stimuli at fixed intervals, resulting in a slower equivalent presentation rate for oddball stimuli, typically around 1 Hz.

e.g., S S S S S O S S S S S O S S S S S O S S S S S O.

The advantage of this approach in signal processing terms is that the

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analysis can focus on particular frequencies in the EEG which include only a small proportion of the broadband ‘noise’ associated with ongoing activity. Traditional ERPs include signals spread across a wide range of frequencies which can include both the signal of interest and a large proportion of the ongoing noise. The FPVS-oddball paradigm examines only the exact frequencies of the standard and oddball presentation, which are defined *a priori*, e.g. at 3 and 0.6Hz. Noise in neighbouring frequencies does not affect the signal of interest and can be ignored, consequently providing very high SNRs (see Norcia, et al 2015a for a review).

To date the approach has been used most commonly in studies of face detection and discrimination (Dzhelyova et al., 2017; Dzhelyova and Rossion, 2014; Liu-Shuang et al., 2014; Liu-Shuang et al., 2016; Rossion, 2014; Rossion et al., 2015; Xu et al., 2017); but has also proven successful in probing low-level visual processing (e.g., orientation encoding (Heinrich et al., 2009); and basic lexical representations (e.g., word/non-word discrimination; Lochy et al., 2015). Recently the approach was also adapted to measure higher level cognitive processing, i.e. abstract semantic categorisation. The spontaneous ability to differentiate between rapidly presented images on the basis of semantic, rather than perceptual properties, was reliably detected in all individual subjects in 1–2mins of EEG recording time (Stothart et al., 2017).

Given the demonstrated success and increasing use of FPVS in reliably measuring perception and cognition in single subjects, our attention turns to a cognitive process of fundamental importance; memory. ERPs have previously been used to study sensory memory via the Mismatch Negativity (MMN) and P3 responses (e.g. Pekkonen et al., 2001; Pekkonen et al., 1994; Polich, 2007), as well as being extensively used to explore models of recognition and working memory via the N250r and FN400 (see Perez and Vogel, 2011; Wilding and Ranganath, 2011 for comprehensive reviews). The current study aims to adapt the FPVS technique to provide an objective measure of visual recognition memory, detectable in minutes, which requires no behavioural recognition response or comprehension of the task. There are many clinical situations in which such an objective covert/implicit measure of recognition memory would be invaluable, e.g. in the early diagnosis of dementia and the assessment of cognition in disorders of consciousness and language where subjects are unable to give a verbal or behavioural response.

To investigate the utility of an FPVS-oddball paradigm for measuring recognition memory, participants completed three conditions; pre-FPVS encoding, repetition and control. In the pre-FPVS encoding condition, oddball images were viewed in an encoding task before the FPVS phase. We predicted higher power at the oddball presentation frequency (0.6Hz) and its harmonics reflecting implicit recognition of the encoded objects. The number of unique oddball stimuli was low to ensure successful recognition, making it necessary to present the images multiple times during the stimulation sequence. This raised the possibility that subjects would learn during the stimulation sequence to recognise

oddball images. To control for this effect of repetition the oddball images in the repetition condition were also presented multiple times but were not previously viewed or encoded. Any increase in power at the oddball presentation frequency (0.6Hz) and its harmonics in this condition could be attributed to online recognition of repeating stimuli during the trial. The control condition consisted of entirely novel stimuli and we predicted no increase in power at 0.6Hz or its harmonics.

2. Method

2.1. Subjects

21 adults (aged 18–38, mean age 23 (± 6), 7 males) gave consent to participate in the study. They were recruited from the University of Bath student population and declared themselves to be in normal health, scored within the normal range on the Addenbrookes Cognitive Exam iii (mean total score = 92 ± 6) and had normal or corrected-to-normal vision. Ethical approval for our procedures were obtained from the University of Bath Psychology Ethics Committee. Subjects provided written informed consent before participating and were free to withdraw from the study at any time.

2.2. Stimuli

Images were selected from the Bank of Standardised Stimuli v2.0 (Brodeur et al., 2014) a previously validated set of 1,468 high quality colour images. All images were 512x512 pixels, 96dpi, subtending 10° visual angle. Importantly each image was only used once, i.e. as a standard, oddball or foil. An example of the images is provided in Fig. 1.

2.2.1. Standards

Standard stimuli (mean image intensity of 0.82 (SD 0.28)), were randomly selected and varied across subjects. Each image was only presented once, with 416 unique images used in the pre-FPVS encoding and repetition conditions, and 520 in the control condition.

2.2.2. Oddballs

Eight oddball stimuli (mean image intensity of 0.82 (SD 0.25)), were pre-selected for the pre-FPVS encoding and repetition conditions, and were consistent across subjects. Equal numbers of natural and non-natural objects were pre-selected in order to ensure no systematic semantic categorical difference between standards and oddballs.

2.2.3. Foils

For the pre-FPVS encoding task two alternative forced choice (2AFC) task eight images were pre-selected as foils (mean image intensity = 0.77 (SD 0.29)) and were consistent across subjects. For the post-FPVS recognition tasks 16 images (mean image intensity of 0.80 (SD 0.29))

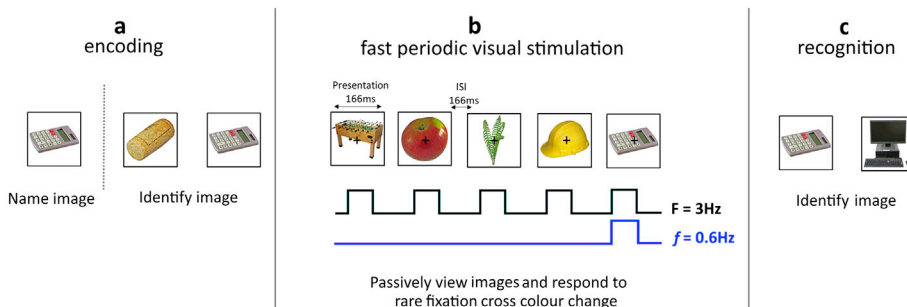


Fig. 1. Pre-FPVS encoding, FPVS and recognition procedures.

a) Pre-FPVS encoding condition only - Subjects named out loud the image, then identified the image in a 2AFC discrimination task paired with previously unseen image (foil).

b) A base frequency F is elicited in response to the presentation of every image at 3Hz, the oddball response f is elicited only to previously remembered images. Subjects attended to the fixation cross and pressed a key when the cross turned red in 10% of randomly selected standard images. Black and blue lines indicate the hypothesised neural response.

c) Subjects identified previously seen oddballs and a randomly selected subset of standard images in a 2AFC discrimination task. Previously seen images were presented alongside novel, previously unseen images (foils).

were randomly selected as foils for the pre-FPVS encoding, repetition and control conditions and varied across subjects.

2.3. Procedure

Subjects completed three conditions; pre-FPVS encoding, repetition and control. In the pre-FPVS encoding condition oddball stimuli were viewed prior to, and repeated during, the FPVS task. In the repetition condition oddball stimuli were not viewed prior to, but were repeated during, the FPVS task. In the control condition subjects viewed a stream of novel stimuli with no oddballs or repetition. Subjects were seated 70 cm from the stimulus monitor.

2.3.1. Pre-FPVS encoding

In order to facilitate the successful encoding of oddball stimuli in the pre-FPVS encoding condition, subjects viewed the 8 pre-selected oddball images centrally for 3s and were asked to name the object out loud. The image was then presented alongside a foil, and the subject was asked to indicate using the left and right arrow keys which image they had just seen. The location of the previously seen oddball images was pseudo-randomised to ensure equal presentations to the left and right of the screen. The subject could not move on to the next image until they had provided the correct response. The purpose of naming the object out loud, and then making a discriminatory choice about the object was to strengthen the encoding of the object, as the depth of processing has been repeatedly demonstrated to be critical to successful encoding (see Brown and Craik, 2000 for a review). Subjects then immediately completed the FPVS task.

2.3.2. Fast periodic visual stimulation

Subjects were instructed to maintain their gaze on a central fixation cross and to press a key in response to the fixation cross turning red, which occurred on 10% of sequences and lasted the duration of the sequence (1.66s). This task was included to avoid lapses of attention that might otherwise exist in a purely passive task. Accuracy and reaction times for this task were recorded. Images were presented in sequences of five images, with the first four images being selected from the standard category and every fifth image from the oddball category. Images were presented onscreen for 166 ms with an inter-stimulus interval of 166 ms. An example of this sequence is presented in Fig. 1b. This design elicits two distinct steady state responses. The standard presentation frequency of 3Hz, and the oddball presentation frequency of 0.6Hz. Each standard stimulus was randomly sampled from the standard image pool and only presented once. Oddball stimuli in the pre-FPVS encoding and repetition conditions were presented 13 times each in a pseudo-random order that ensured no consecutive presentations. In total 520 stimuli were presented in one trial lasting 173s. In the control condition 520 previously unseen novel stimuli were presented in a random order. The order of the conditions was counterbalanced across subjects.

2.3.3. Recognition task

Immediately following each of the three FPVS tasks subjects completed a recognition task in which they were presented with a 2AFC discrimination task in which 16 images previously seen during the FPVS task were paired with 16 foils (novel, previously unseen images), and the subject was asked to indicate which image they had seen during the experiment. In the pre-FPVS encoding and repetition conditions the previously seen images were the eight oddball stimuli and a random selection of eight standard stimuli, and in the control condition they were a random selection of 16 standard stimuli.

2.4. EEG recording

EEG signals were sampled at 1000Hz from 65 channel HydroCel Geodesic Sensor Net electrodes using a GES 400 system (Electrical Geodesics Inc; EGI, Eugene, OR, USA), with a common Cz reference

and online low-pass filtered at 250Hz. Impedances were below 50 k Ω . Recordings were analysed offline using Brain Electrical Source Analysis software v5.3 (BESA GmbH), Matlab (Mathworks Inc.) and the Fieldtrip toolbox (Oostenveld et al., 2011). Blinks and eye movement artifacts were corrected using BESA automatic artifact correction (Berg and Scherg, 1994).

2.5. EEG analysis and steady state response

Data were re-referenced offline to a common average reference, downsampled to 256Hz, and two electrooculogram electrodes were excluded from further analysis. To avoid aliasing artifacts an 85Hz 24 db zerophase lowpass filter was applied. The steady-state response was calculated according to the procedures described in Stothart et al. (2017). Epochs from 0 to 173s around trial onset were defined for each condition. This epoch length represents an integer number of cycles (104) of the oddball stimulus (0.6Hz) ensuring that a frequency bin corresponding to the exact oddball frequency and its harmonics, including the standard frequency (3Hz), were created. The frequency resolution was 0.0057Hz. Epochs were first linearly de-trended and the DC component was removed. As we used single epochs of a long duration, visual inspection revealed occasional instances of gross artifacts, e.g. large physical movement artifacts. Any artifact $\pm 250\mu\text{V}$ was removed from the data and replaced with zeros. To avoid discontinuities in the remaining data, data on either side of any removed section was tapered to zero using half a hanning window over 670 points of data. Across subjects, the mean percentage of data removed by this procedure was 0.4% ($SD = 0.6\%$) in the pre-FPVS encoding condition, 0.6% ($SD = 1.9\%$) in the repetition condition and 0.5% ($SD = 0.9\%$) in the control condition. For each subject and each electrode, amplitude was computed on these windows using the fast Fourier transform (FFT). SNR was then calculated by dividing the amplitude in each frequency bin by the mean amplitude of surrounding bins within a $\pm 0.10\text{Hz}$ range (17 frequency bins) (e.g. Alp et al., 2016; Srinivasan et al., 1999; Stothart et al., 2017) excluding the immediately adjacent bins (first neighbouring bin on each side). Excluding the immediately adjacent bins from this correction meant that the amplitude correction was less likely to include any spread of the signal to proximal frequency bins (e.g. for 0.6Hz adjacent bins were 0.5941 & 0.6059Hz).

Previous research has shown a robust SSVEP response to the oddball frequency and many of its harmonics (Norcia et al 2015b; Rossion et al., 2015) with oddball detection more reliably and accurately measured when including the harmonics of the oddball response (e.g. Stothart et al., 2017). Consequently the SNR was calculated for 2 values: the standard frequency F (3Hz) and the mean of the oddball frequency and significant harmonics f_+ . To identify which harmonics to include in the calculation of f_+ , group Z scores were calculated for each harmonic (based on the global average of all electrodes averaged across the three conditions) relative to the neighbouring frequency bins within a $\pm 0.10\text{Hz}$ range. This identified the highest significant harmonic ($Z > 1.645$) at 7.2Hz, therefore for further analyses f_+ was always calculated as the mean SNR of 0.6, 1.2, 1.8, 2.4, 3.6, 4.2, 4.8, 5.4, 6.6 and 7.2Hz. These three values were calculated for each subject and electrode for all three conditions. Using Fieldtrip software (Oostenveld et al., 2011), the difference in f_+ between condition pairs (pre-FPVS encoding vs control, pre-FPVS encoding vs repetition, repetition vs control) was statistically assessed across all electrodes using cluster-based permutation analysis (Maris and Oostenveld, 2007) with 10,000 permutations. Initial cluster analysis at cluster formation $\alpha < 0.05$ revealed one large cluster which included all electrodes, this is one weakness of the cluster permutation approach highlighted recently by Mensen and Khatami (2013). Therefore to identify the strongest areas of activity on the scalp, the entry α was reduced to < 0.01 (one-tailed).

2.6. Presentation durations required to measure the steady state response

To investigate the duration/amount of visual stimulation needed to detect a significant difference between conditions we created 17 different duration windows ranging from 10 s of data up to 170 s of data that increased in 10 s increments. For each duration, we compared the mean Z score of f_+ from the highest cluster electrode for the pre-FPVS encoding and repetition conditions versus the highest cluster electrode from the control condition. For each subject the electrode showing the maximum response was allowed to vary, but was restrained to the original group cluster (pre-FPVS encoding vs control) electrodes ($n = 40$), allowing us to account for individual differences in the topography of the f_+ response. Condition differences across increasing durations of data were assessed using a permutation procedure (Blair and Karniski, 1993) in which a reference distribution was calculated using the summary statistic t_{max} (the largest absolute value of t for all duration windows tested in each permutation). All comparisons in the original data were compared to the tails of the maximum values in the reference distribution.

2.7. Pre-registration, data and code sharing

The study was pre-registered on the Open Science Framework (<https://doi.org/10.17605/OSF.IO/HV49F>). We have also made the stimulus presentation code, data and analysis code freely available on the Open Science Framework (https://osf.io/dpmec/?view_only=8035aa10b781425390b02d5db11c7aa9).

3. Results

3.1. Behavioural performance

3.1.1. FPVS fixation cross colour change detection

Subjects did not show significant statistical differences across conditions in their mean reaction time (Pre-FPVS encoding: $M = 452$ ms ($SD = 122$); Repetition: $M = 476$ ms ($SD = 161$); Control: $M = 475$ ms ($SD = 112$); $F(2,40) = 0.29$, $p = 0.753$, $np2 = .014$) or accuracy (Pre-FPVS encoding: $M = 98\%$ ($SD = 0.7$); Repetition: $M = 99\%$ ($SD = 0.6$); Control: $M = 93\%$ ($SD = 3.3$); $F(2,40) = 2.45$, $p = 0.09$, $np2 = .109$) to fixation cross colour changes.

3.1.2. Post-FPVS recognition task

In the pre-FPVS encoding condition all subjects performed at ceiling in correctly identifying the oddball images they had been asked to remember. In the repetition condition performance was high with 15 participants at ceiling and six participants showing errors (see Fig. 2). Wilcoxon Signed-Ranks Test indicated that the median accuracy was statistically significantly higher in the pre-FPVS encoding than the repetition condition, $Z = -2.27$, $p = 0.023$. There was no significant correlation of oddball recognition accuracy with f_+ in the repetition condition ($r(20) = -0.03$, $p = 0.884$), correlations could not be calculated in the pre-FPVS encoding condition given the ceiling effect in the behavioural data.

Recognition of images presented once as standard stimuli was lower than oddball images, reflecting the benefit of pre-FPVS encoding and repetition versus the single viewing of a stimulus (see Fig. 2). The difference in standard stimulus recognition accuracy across the three conditions was examined using a 1-way repeated measures ANOVA (pre-FPVS encoding, repetition, control). There was a main effect of condition ($F(2,40) = 8.50$, $p = 0.001$, $np2 = .298$). Post hoc tests using the Bonferroni correction revealed that recognition was lower in the control compared to pre-FPVS encoding condition (mean difference = -22.0 , $p = 0.001$), and the repetition condition (mean difference = -17.9 , $p = 0.008$). There was no significant difference between accuracy in the pre-FPVS encoding and repetition conditions (mean difference = 4.17 , $p = 0.432$).

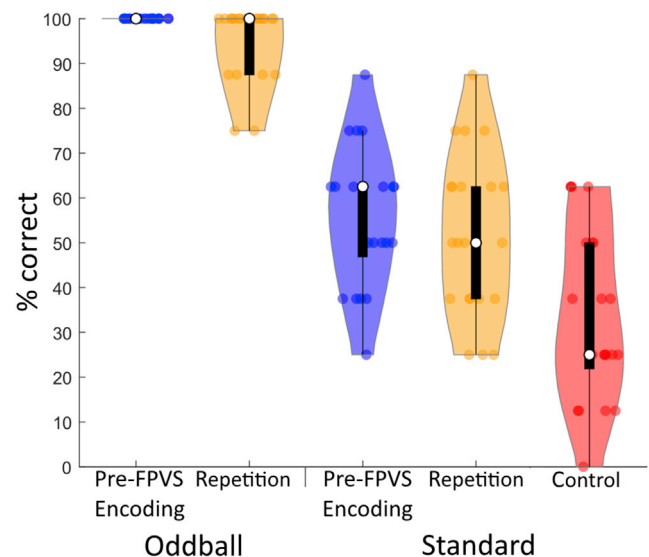


Fig. 2. Violinplots illustrating % accuracy for the recognition tasks. Scores reflect the correct recognition of either an oddball or standard stimulus compared to a foil in a 2AFC. Tukey boxplots reflect the median and inter-quartile ranges, width of the violinplots reflects kernel density estimated using Matlab's ksdensity function.

3.2. Recognition memory oddball responses

Fig. 3 shows the global average amplitude and SNR in response to the three conditions. Clear oddball responses were observed at the oddball frequency f and the harmonics up to $7.2\text{Hz } f_+$ in the pre-FPVS encoding condition. A one-way repeated measures ANOVA tested the effect of condition (pre-FPVS encoding, repetition, control) on the strength of f_+ in the global average. There was a main effect of condition on f_+ SNR ($F(2,40) = 28.35$, $p < 0.001$, $np2 = .586$). Post hoc tests using the Bonferroni correction revealed that f_+ was larger in the pre-FPVS encoding compared to repetition condition (Mean difference = 0.08 , $p = 0.03$, Cohen's $d = 0.68$), and compared to the control condition (Mean difference = 0.22 , $p < 0.001$, Cohen's $d = 1.52$). f_+ was larger in the repetition compared to the control condition (Mean difference = 0.13 , $p < 0.001$, Cohen's $d = 1.39$).

3.3. Cluster permutation analyses

Having established clear differences between the three conditions in data averaged across the scalp, cluster permutation analyses were subsequently conducted to determine the topographic maxima of the oddball responses.

3.3.1. Pre-FPVS encoding vs control

Cluster permutation analysis revealed a 40 electrode cluster with differences in the SNR of f_+ strongest at the vertex and lateral occipital areas, cluster $p = 0.0009$ see Fig. 4.

3.3.2. Pre-FPVS encoding vs repetition

Cluster permutation analysis revealed two electrode clusters with differences in the SNR of f_+ . A frontal left cluster of two electrodes, cluster $p = 0.04$, and a four electrode central left cluster, cluster $p = 0.01$, see Fig. 4.

3.3.3. Repetition vs control

Cluster permutation analysis revealed two electrode clusters with differences in the SNR of f_+ . A frontal right cluster of three electrodes, cluster $p = 0.00019$, and a 15 electrode occipital cluster, cluster $p = 0.01$, see Fig. 4.

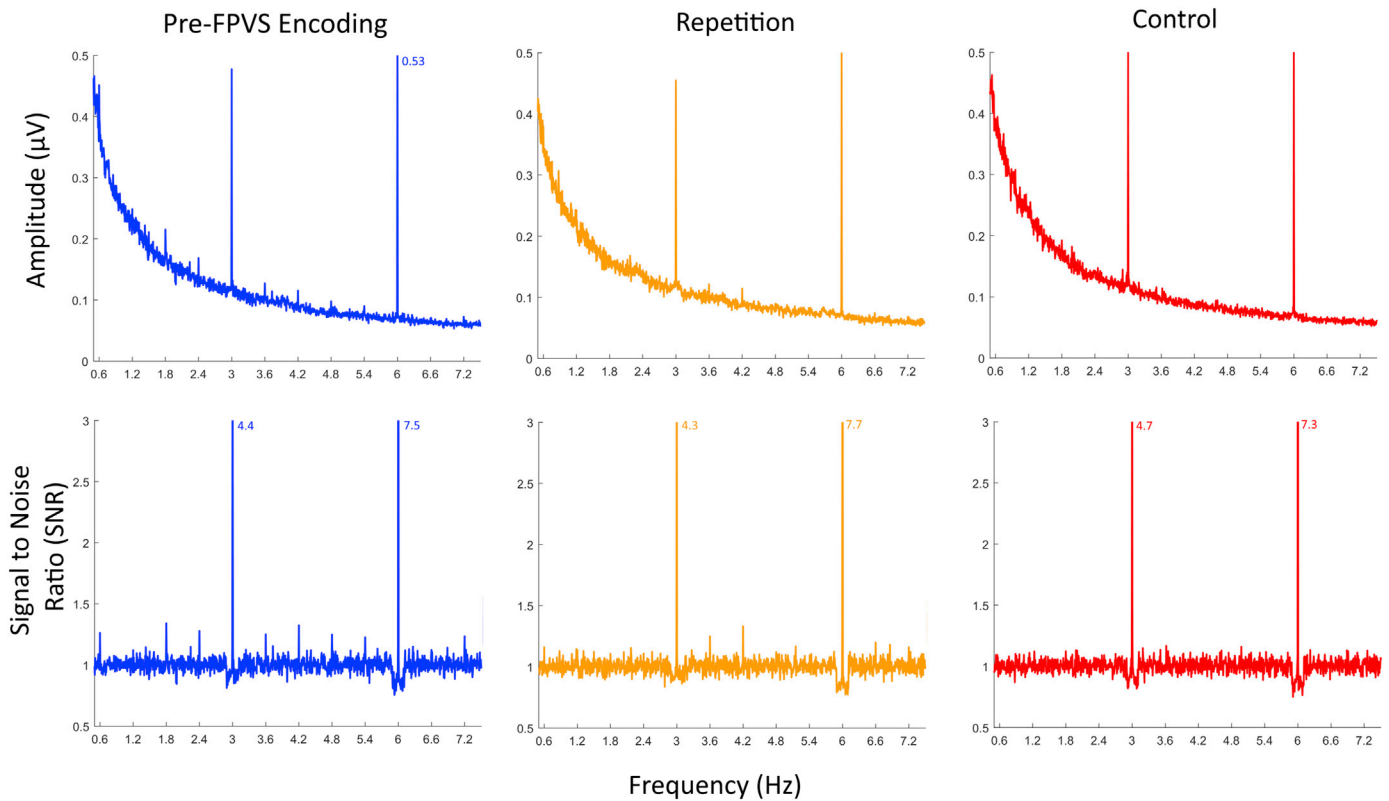


Fig. 3. Spectral plots represent the amplitude and signal to noise ratio for the three conditions, averaged across all electrodes ($n = 63$) and all subjects ($n = 21$). Values for F are provided when larger than y axes maxima.

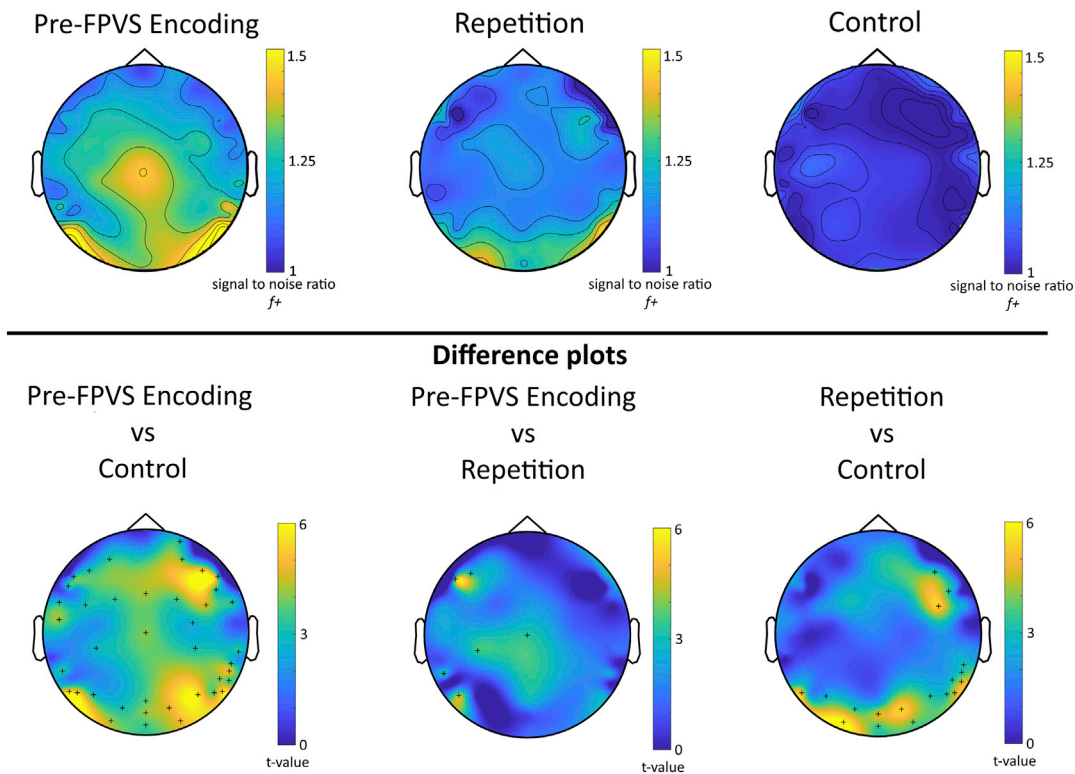


Fig. 4. Topographic plots indicate the SNR of f_+ (mean of 0.6, 1.2, 1.8, 2.4, 3.6, 4.2, 4.8, 5.4, 6.6 and 7.2Hz) in the three conditions. Difference plots illustrate the electrodes identified by cluster permutation analysis as showing significant differences in the SNR of f_+ between the conditions.

An exploratory and descriptive analysis of the data was conducted in the time domain. A negative deflection in the evoked responses to the pre-FPVS encoding and repetition conditions that was absent in the control conditions was observed approximately 200 ms after the onset of the oddball stimulus and strongest at right parieto-occipital electrodes. The timing and topographic distribution of this difference closely resembled a classic visual MMN response, see Supplementary Information, Fig. S1.

3.4. Neuro-behavioural correlations

To investigate the relationship between individual differences in f_+ and behavioural performance, subjects were dichotomised as responders, $n = 9$, and non-responders, $n = 12$, on the basis of their z-scores in section 3.5. Independent samples t-tests indicated no significant differences between the two groups in recognition task accuracy or detection accuracy/speed of fixation cross colour changes ($p > 0.05$ for all comparisons).

3.5. Single subject effects

Having established the clear presence of pre-FPVS encoding and repetition oddball responses at the group level we examined the strength of the effect at the single subject level. The SNR of f_+ was selected from the cluster electrode that showed the largest response per subject in the pre-FPVS encoding, repetition and control conditions. The electrodes were restricted to the 40 electrodes identified in the pre-FPVS encoding vs control group permutation analysis, but importantly were allowed to vary from individual to individual (see Fig. 5a). P8 showed the largest

oddball response for the greatest number of subjects in both pre-FPVS encoding and repetition conditions with a trend for greater variability in the pre-FPVS encoding condition. Fig. 5b shows the individual SNR values for the three conditions, Fig. 5c shows individual Z scores for the pre-FPVS encoding, repetition and control conditions. 20 out of 21 subjects showed a higher Z score for f_+ to the pre-FPVS encoding condition compared to the control condition, and this was replicated in data averaged across all electrodes (see Supplementary Information, Fig. S2). 9 out of 21 subjects showed a pre-FPVS encoding condition Z score greater than 1.645 ($p < 0.05$, one-tailed). Given that SNR and Z-scores were consistently higher in the pre-FPVS encoding versus the control condition, we applied a binary classification approach to see whether the SNR signal for f_+ could predict whether a trial was from the pre-encoding FPVS or the control condition. To create more data to train and test the learning algorithm, we segmented each participant's original 173-second trial into three 55-second trials. We then used regularized logistic regression (implemented with the Matlab function lassoglm) with 10-fold cross validation. Classification accuracy of condition was 83% ($b_0 = -4.669$; $b_1 = 3.769$; $\lambda = 0.013$; Deviance = 140.299; SE of Deviance = 9.298; LambdaMinDeviance = 0.0120).

Fig. 5d illustrates the individual harmonics that contribute to each subject's f_+ score. It is clear that for every subject the calculation of f_+ (i.e. the mean of all harmonics significant at the group level) included non-significant harmonics and that for subjects with an f_+ Z score of less than 1.64 there were still multiple significant harmonics. While f_+ may be a reliable measure for group level comparisons, it does not fully capture inter-individual variability in the strength of oddball and harmonic responses.

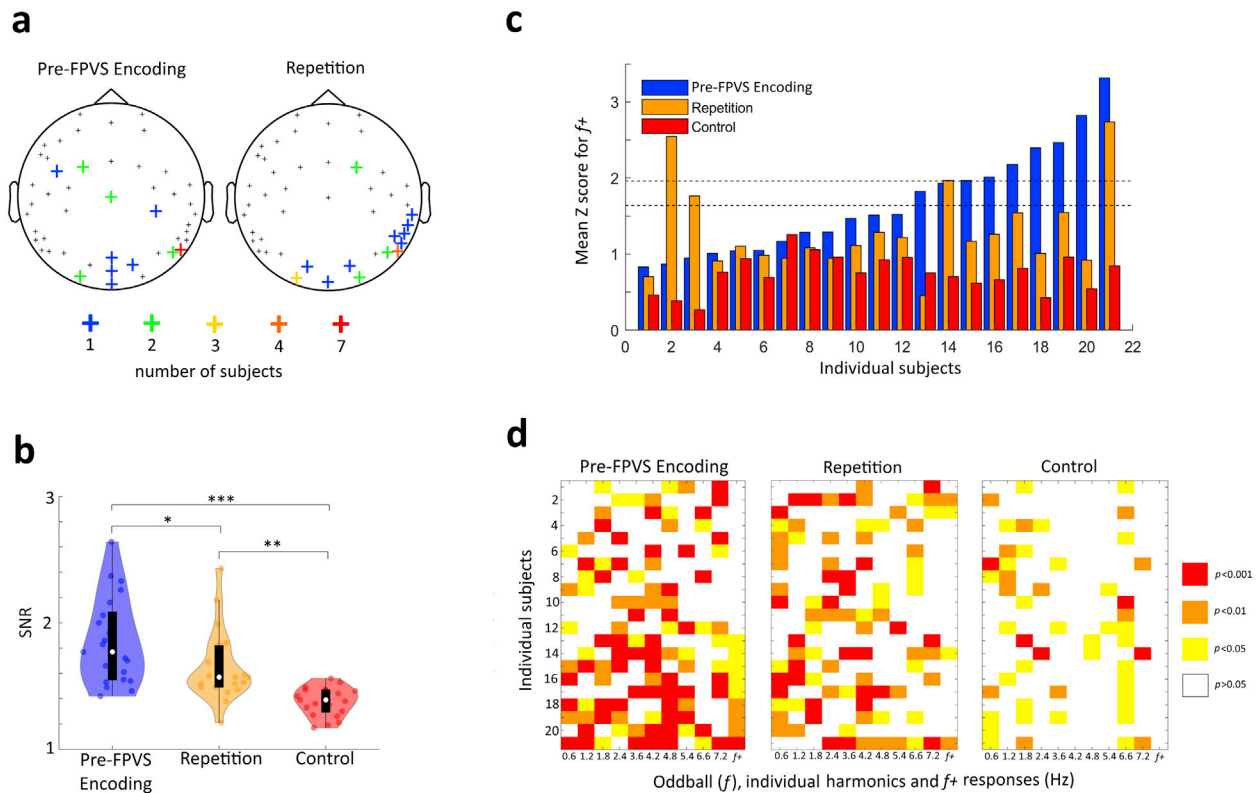


Fig. 5. a) Scalp plot indicating cluster electrodes that showed the largest f_+ SNR value for individual subjects. b) Violinplots illustrating SNR of f_+ for each subject at the cluster electrode that showed the largest value for each condition. Tukey boxplots reflect the median and inter-quartile ranges, width of the plots reflects kernel density estimated using Matlab's ksdensity function. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. c) Z scores of f_+ for individual subjects for the three conditions calculated at the cluster electrode that showed the largest SNR value. Dotted lines indicate $z = 1.645$ ($p < 0.05$, one-tailed) and $z = 1.96$ ($p < 0.05$, two-tailed) thresholds. d) Z scores of f_+ , individual harmonics and f_+ for each subject at the cluster electrode that showed the largest f_+ Z score for each condition. Colours represent one-tailed Z-score thresholds. Red: $Z > 3.09$, $p < 0.001$, orange: $Z = 2.32-3.089$, $p < 0.01$, yellow: $Z = 1.64-2.319$, $p < 0.05$, white: $Z \leq 1.639$, $p > 0.05$.

3.6. Presentation durations required to detect the memory oddball response

Fig. 6 shows condition differences across increasing durations of analysed data ranging from 10 s to 170 s in epochs increasing in size by 10 s increments. Pairwise comparisons between conditions were undertaken for all durations corrected for multiple comparisons (see Methods section 2.6). As indicated in Fig. 6, mean Z scores for f_+ were highest in the pre-FPVS encoding condition, followed by the repetition condition and were lowest in the control. Group Z scores were significantly increased in the pre-FPVS encoding condition compared to control at all durations of 50 s and over. The difference between pre-FPVS encoding and repetition conditions was not significant at any duration length, and similarly repetition did not differ from control for any duration length. This analysis does not reveal the time-course of the effect, but does indicate that the minimum duration of stimulus presentation/data needed to observe a reliable group difference between pre-FPVS encoding and control was 50 s.

It should be noted that the Z scores for the control condition in Figs. 6 and 5d were significantly above zero, this is likely due to the selection of bespoke electrode showing the maximum response for each condition. Our hypothesis is that activity at f_+ frequencies is comprised of signal + noise for the pre-FPVS encoding and repetition conditions, and only noise in the control condition (as there is no meaningful oddball stimulus). By selecting the maximum electrode in the control condition, as opposed to simply selecting the same one as in the pre-FPVS encoding or repetition condition, we are giving the control condition an equal statistical chance for false positive results driven by random noise. This also explains why in Fig. 5d there are multiple significant individual harmonics in the control condition. It is clear in both cases that although this results in Z scores greater than zero, this effect plateaus and large experimental effects remain.

4. Discussion

4.1. Summary of results

Clear oddball responses were observed in response to the presentation of previously seen and remembered images (pre-FPVS encoding condition) and statistically significant at the group level in under 1 min of EEG recording. Oddball responses were also observed in response to previously unseen and repeatedly presented oddball stimuli (repetition condition) but were significantly weaker than those observed in the pre-

FPVS encoding condition. No oddball responses were observed in response to novel previously unseen images (control condition).

Cluster permutation analyses revealed the strongest oddball responses at the vertex and occipito-parietal electrode sites. Right hemisphere frontal electrodes also showed differences in both the pre-FPVS encoding vs control and repetition vs control comparisons. Examination of individual differences revealed a larger pre-FPVS encoding condition f_+ response in 20 out of 21 subjects compared to the control condition. Only 9 out of 21 subjects showed a statistically significant f_+ Z score due to the high degree of inter-subject variability in the frequency and number of harmonics that contributed to f_+ . Behavioural recognition measures demonstrated that subjects had learned the oddball images successfully, with performance at ceiling in the pre-FPVS encoding condition and near ceiling in the repetition condition. Overall the data demonstrate that the FPVS technique can be successfully adapted to provide an objective measure of visual recognition memory, detectable in minutes and requiring no behavioural response or comprehension of the task.

4.2. Adaptation to memory

This is the first study to demonstrate the adaptation of the FPVS technique to the measurement of visual recognition memory and builds on our previous work in which we demonstrated that FPVS could be used to measure semantic categorisation (Stothart et al., 2017). It is becoming clear that FPVS is a highly flexible and adaptable method, capable of capturing higher level cognitive as well as perceptual and attentional processes. Importantly, we have again demonstrated that the FPVS technique is sensitive to implicit processing as subjects were instructed to respond to an orthogonal attentional task (detection of occasional fixation-cross colour change) but were not directed towards the main experimental distinction of remembered or repeated images. Explicit recognition responses were collected following the FPVS task and demonstrated that subjects were easily able to recognise the oddball images in both the pre-FPVS encoding and repetition conditions. The recognition of standard stimuli that had been viewed just once during the FPVS task was near chance in the pre-FPVS encoding and repetition conditions, and surprisingly lower than chance in the control condition. There was a non-significant trend for poorer target identification in the orthogonal fixation cross colour change detection task during the control condition, which although still high at 93% suggests that subjects' engagement with the task may have been lower, providing a possible explanation for poorer recognition of control stimuli.

4.3. The role of encoding and stimulus repetition

The effect of pre-FPVS encoding + repetition was stronger than repetition alone when compared in the scalp average, cluster permutation testing and also in the time duration analyses. However, an oddball response was observed in the repetition condition, indicating that subjects learned "on-line" during the stimulation sequences to recognise repeatedly presented oddball stimuli. Oddball responses to both the pre-FPVS encoding and repetition conditions showed most strongly at occipito-parietal electrodes, with additional activity in the pre-FPVS encoding condition at fronto-central areas, potentially reflecting recognition memory specific evoked responses such as the N250r and FN400 (Wilding and Ranganath, 2011). Cluster permutation testing highlighted central-left hemisphere electrodes as showing significant differences in activation between the pre-FPVS encoding and repetition conditions.

The presence of oddball responses in both conditions, and their topographic differences, lead us to conclude that the oddball responses observed in the pre-FPVS encoding condition were a product of two memory processes. The first reflects a match between the stimulus and a stored representation of that item, i.e. the core underlying process responsible for the experience of familiarity in models of recognition memory (Norman and O'Reilly, 2003). Crucially the oddball responses

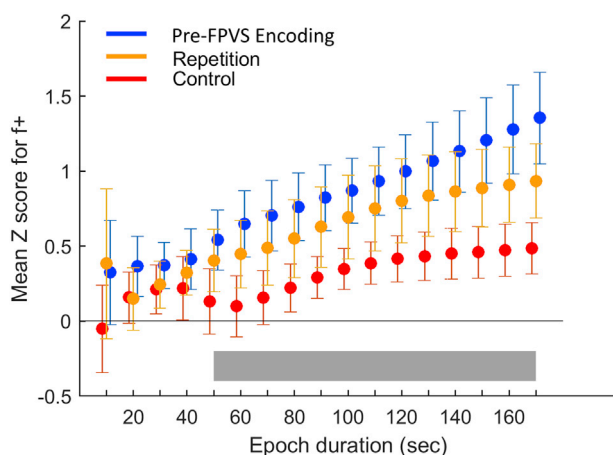


Fig. 6. Group level changes in mean Z score of f_+ as a function of increasing duration of data analysed measured at the cluster electrode that showed the largest value for each condition. Error bars denote 95% CI, the grey bar indicates the durations at which statistical comparison of Pre-FPVS encoding vs Control conditions showed significant differences. No other pairwise differences reached statistical significance at any duration analysed.

are an objective form of recognition given the absence of task instructions or requirement to consciously recognise or recall the stimulus. The second is driven by repetition effects and may be explained through a range of theoretical interpretations. Cumulative exposure to the stimuli in the case of the pre-FPVS encoding condition would strengthen the initial encoding, whereas in the repetition condition the initial repetitions may act as a passive proxy encoding phase, with subsequent repetitions strengthening this online encoding. As the number of repetitions of each stimulus increases it may also result in a form of statistical learning (Mandikal Vasuki, et al, 2017; Saffran, 2003) as the subject begins to implicitly classify the stimuli into “repeating” vs “non-repeating” stimuli. This statistical learning could occur either through transitional probabilities (it is more likely that each image is followed by a novel image than a repeated one) or chunking (fourth standard is always followed by a repeated object) (Dehaene et al., 2015).

This establishment of discrete categories would then result in a classic MMN response (Näätänen and Michie, 1979)/predictive coding error signal (Stefanics et al., 2015), with the regularity of the standard novel stimuli being interrupted by the occurrence of a repeated stimulus. In line with this the topographic distribution of the oddball responses showed activity at both the vertex and right frontal electrodes, previously shown to be areas reflecting the activation of frontal neural sources in visual change detection (Deouell, 2007; Hedge et al., 2013). Exploratory time domain analysis also showed a visual MMN like response following the oddball stimuli (see Supplementary Information, Fig. S1).

4.4. The advantages of measuring recognition memory with FPVS

4.4.1. Theoretical development

The implicit and objective nature of this measure is of potential significance in further measuring and investigating recognition memory. Not only does it provide an index of recognition that might be usefully contrasted with subjective report, but the oddball response may also be amenable to experimental manipulation in a way that can inform theories of recognition memory (e.g. Curran et al., 2006; Jacoby and Dallas, 1981; Yonelinas, 2002). The FPVS task elicited recognition-related responses without instructing subjects to remember making it uniquely well-suited to examining indirect/implicit memory effects, however we would suggest that its utility is not limited to this scenario.

Dual-process models of recognition memory propose that people may recognise a stimulus on the basis of conscious recollection or familiarity (for a review see Yonelinas, 2002), processes that are typically dissociated by subsequent mnemonic ratings (e.g. remember/know distinctions). Subjects did not provide remember/know distinctions in the current study, however the low number of encoded images and the ceiling performance in the subsequent behavioural recognition task would suggest that people were likely to be able to recollect the images. Future studies could manipulate the strength of initial encoding (by using, for instance, a passive encoding paradigm and a higher number images) to investigate whether oddball related responses and their associated topography may be dissociated on the basis of subsequent recollection. Indeed, the topographic difference between the pre-FPVS encoding and repetition conditions reported in this experiment is consistent with the plurality of familiarity processes (Lucas et al., 2012) and distinct neural processes underlying repetition and recognition effects. There is also the opportunity to further investigate perceptual vs semantic drivers of familiarity (through the use of perceptually or semantically related oddball stimuli) and statistical learning (through the manipulation of oddball repetition).

4.4.2. Experimental utility

The approach has many advantages over ERPs from a methodological standpoint. The *a priori* definition of frequencies of interest means that task irrelevant neural activity and random noise occurring at broadband frequencies can be ignored. The subsequent gain in SNR means that recording times can be remarkably short and increases the viability of

spatially modelling the responses in order to examine the neuroanatomical correlates of recognition memory processes (Aggleton and Brown, 1999). Electrode selection, as always, remains a potential source of experimenter bias, however we have demonstrated that effects are detectable both when averaging across all electrodes (completely removing electrode selection from the process) and when using data driven electrode selection through a cluster permutation approach.

The oddball responses observed likely reflect previously documented event related neural responses to familiarity, repetition and non-target oddball detection, e.g. the N250r, FN400 and vMMN (Curran et al., 2006, p. 250; Polich, 2007; Rugg et al., 1998; Tales et al., 1999; Vilberg et al., 2006). The clearest difference between conditions in the exploratory time domain analysis (see Supplementary Information, Fig. S1) was between 160 and 250 ms at parieto-occipital electrode sites and closely resembled a classic visual MMN response. The N250r is larger to familiar images and to those that have undergone extensive repetition (Itier and Taylor, 2004; Tanaka et al., 2006). This is consistent with the pattern of pre-FPVS encoding > repetition > control in the current study. The FN400 is observed on frontal electrodes in old/new contrasts and is consistent with the pattern of frontal activity observed in both the pre-FPVS encoding and repetition conditions. The fast presentation of images in the current study make it difficult to dissociate these event related components, however the examination of individual differences in ERP locations and strength would be a valuable pairing with FPVS in future studies in order to explore the functional relevance of the different topographical maxima of $f+$ observed in the current study.

4.4.3. Clinical utility

The short recording times and implicit nature of the task gives the FPVS approach significant advantages over ERPs in terms of developing clinical tools. For example, visual recognition memory is reliant on medial temporal lobe structures such as the perirhinal cortex and the hippocampus (Squire and Zola-Morgan, 1991), structures that undergo significant atrophy during the course of Alzheimer's disease (AD) (Braak and Braak, 1991; Hyman et al., 1990). The consequences of this structural damage manifest behaviourally in poor recognition memory performance in AD and Mild Cognitive Impairment patients (Barbeau et al., 2004; Didic et al., 2013). Kirwan and colleagues demonstrated using fMRI that activity in the medial temporal lobe correlated with old/new status of a stimulus in healthy control subjects, but was independent of the accuracy of subsequent old/new behavioural ratings (Kirwan et al., 2009). We propose that the FPVS oddball responses observed in the current study may reflect such activity. The FPVS approach could provide a faster, more affordable and practical objective measure of recognition memory than fMRI, and a more reliable and sensitive measure than classic behavioural tasks. Fundamentally behavioural measures require comprehension of the task and the functional and linguistic ability to provide a response. For populations that are not able to meet these demands (e.g. aphasic, altered consciousness and cognitively impaired populations) the FPVS approach shows promise as a useful tool for the assessment of higher-level cognitive function, although it must be noted that the pre-FPVS encoding task would require some adaptation for such use.

4.5. Limitations and future research

Examination of the presence of a statistically significant oddball response at the level of the individual subject showed z-scores above a 1.645 ($p < 0.05$, one-tailed) threshold in 9 out of 21 subjects. This is lower than in previous studies examining semantic categorisation (Stothart et al., 2017) and individual face discrimination (Liu-Shuang et al., 2014). It may be that the stimulation duration in the current study (180s) was sufficiently long to detect group effects but to improve the sensitivity of the method at the individual subject level longer durations may be required. Examination of f and the individual harmonics that were significant at the group level indicated a high degree of inter-subject

variability in the frequency and number of harmonics that contributed to the $f+$ value. This high variability partially explains the low number of individuals showing significant $f+$ Z scores as every subject's score included non-significant harmonics in the calculation of the mean. Furthermore, subjects whose $f+$ score was <1.64 all showed multiple significant individual harmonics. Additionally, it should be noted that a larger $f+$ was observed in the pre-FPVS encoding compared to the control condition in 20 out of 21 subjects, demonstrating the consistency of the experimental effect. Future studies should investigate the role of individual harmonic responses in the characterisation of oddball, and therefore recognition responses. When grouped as responders and non-responders on the basis of these z-scores there were no behavioural performance differences between these subjects. It should also be noted however that this was an under-powered post-hoc analysis, and future studies should compare $f+$ against well-established and sensitive behavioural measures of recognition memory.

There are many adjustable parameters (e.g. stimulus type, standards: oddball ratios, depth of encoding, number of unique oddballs vs. number of repetitions, selection of different harmonics) that may improve the reliability of this effect at the individual subject level. For example, the current presentation rate of 3Hz was chosen to ensure that subjects had sufficient time in between stimuli (333 ms) for stimulus processing and early recognition processes (e.g. N250r) to occur. Faster presentation rates may improve the strength of the oddball responses as the oddball frequency will be higher and further from the high amplitude noise caused by the 1/f distribution of the human EEG spectrum. Conversely slower presentation rates may cause less stimulus-to-stimulus interference and allow for more discrete recognition responses. Implementing alternative oddball designs, such as roving paradigms, would also help to control for low level visual differences between standard and oddball stimuli. The current study provides proof of principle for the approach and points the way for future empirical studies.

4.6. Summary and conclusions

In summary we present a new method for objectively measuring implicit visual recognition memory that is fast to implement and requires no comprehension of the task or behavioural response. Fundamentally this is a method that can measure recognition memory in isolation, free from the confounds and variability of behavioural recognition tasks.

Author contributions statement

George Stothart; Conceptualisation, Methodology, Software, Formal Analysis, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Laura J Smith; Methodology, Software, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing.

Alexander Milton; Software, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization.

Acknowledgements

This work was supported by BRACE, registered charity no:297965, grant number BR17/58.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuroimage.2020.116628>.

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